Study of Physical Biochemical Antioxidant and Antimicrobial Parameters of Honey in Western Maharashtra.

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1. Introduction:

Honey is the natural sweet substance produced by honey bees from the nectar of plants or from the secretions of living parts of the plants or excretion of plant, sucking insects on the living parts of the plants, that bees collect, transform by combining with the specific substance of their own deposit, dehydrate, store and leave in the honeycomb to ripen and mature. **- Food and Agriculture Organization's Codex Alimentarius Commission.**

Apis dorsata, Apis Florea, Apis cerana indica, Apis mellifera, (These bees include in a Phylum: Arthropoda, Class: Insecta, Order: Hymenoptera, Superfamily: Apoidea, Family: Apidae, and Genus: Apis), *Melipona irridipennis* (Meliporidae) and other different species of honey bees plays important role in the formation of honey.

Honeybees keep honey in their hives and used it for nutrition and health. Regular honey is processed before being bottled, whereas raw honey comes straight from the honeycomb. According to cultural tradition, honey has been widely accepted as food and medicine in all generations since ancient times. In Ayurveda, honey is the base for the preparation of most medicines because it is healthy, has high nutritional value, is easy to digest, produces energy faster, and strengthens the body's immunity. Therefore, honey is called the mother of food.

Honey could be a supersaturated solution of sugar, of which fructose (38%) and glucose (31%) are the major contributors, but it also contains various trace components like enzymes, amino acids, organic acids, carotenoids, Maillard reactions Products, vitamins, minerals, and polyphenols.

The nature of honey depends mainly on the flowering and geographical origin of the plant. Geographical changes reduce the number of flowers in summer or some seasons. In this case, many beekeepers save the bees by giving them sugar water as food. It affects the quality of honey. Additionally, the processing, handling, and storage of honey may influence its composition.

Honey is widely used in medicine. Seeing its benefits, many people see buying honey every day. Many surveys show that people are also buying and selling at higher rates.

Since the COVID-19 epidemic, many people around the world have increased their consumption and preference for honey to boost immunity. Therefore, honey is still in great demand all over the world. To meet the growing demand for this product, many companies are using high-quality fructose syrup to make honey. In short, many are making adulterated honey. So, it is extremely dangerous for health. To avoid this problem nationally and internationally, honey analysis is more important.

Maharashtra has a versatile geographical climatic condition. The state has a tropical monsoon climate; Hot summers begin in March and end with rains in early June. The rich green cover of the rainy season survives the mild winter after the October transition. The highly active nature of the monsoon includes rains and prolonged droughts and floods. It contributes greatly to the instability of the rural economy.

Due to its almost perennial flowing rivers in western Maharashtra, nutritious environment for agricultural crops, green visitation has created a fertile environment for beekeeping.

To give a special place to the producer locally, domestically as well as in the world, to increase the confidence of the producers, to provide good quality honey to the honey consumers. It is very important to check the honey production and its quality.

The aim of the present study is to investigate the Physical, Biochemical, Antioxidant, and Antimicrobial Parameters of honey in Western Maharashtra and to spotlight on comparing the quality of the research results with the standard results and analyses the causes of the quality and quantity of honey.

2. Statement of problem :

“Study of Physical, Biochemical, Antioxidant and Antimicrobial Parameters of Honey in Western Maharashtra”.

3. Conceptual and Operational definitions:

A. Conceptual definition:

- **Beehive:** A mass of six-sided cells made by honey bees in which brood is reared and honey and pollen are stored; composed of two layers united at their bases.
- **Apis mellifera**: The scientific name of a honey bee found in the United States.
- **Colony of Bee:** Group of worker bees, drones, and queen bees developing colonies living together as a family unit in a hive.

B. Operational definition:

- **Beehive:** A man-made home for bees.
- **Honey:** Honey is a sweet, viscous, deep golden liquid food made from the nectar of flowers in the honey sacs of worker bees.
- **Capped:** A thin layer of wax on cells filled with honey.
- **Bee Brush:** A brush or broom used to remove bees from the hive.
- **Western Maharashtra:** Western Maharashtra (Paschim Maharashtra) is a region of the Indian state of Maharashtra, which includes the districts of Sangli, Satara, Kolhapur, Solapur, Pune, and Nashik.
- **Sangli:** One of the district places of Maharashtra.

4. Objectives of Research:

The main objectives of this study are:

A. To collect and preserve samples from different districts in western Maharashtra, India.
B. To investigate the Physical, and Biochemical properties of honey samples.
C. To investigate Antioxidant and Antimicrobial Parameters of Honey.
D. To analyze honey samples for the detection of pesticides, antibiotics, heavy metals, toxic residues, and other adulterants.
E. To determine the standard of honey in Western Maharashtra by comparing research data with standard data.
F. To investigate regional differences within the physical, and Biochemical properties of honey.
G. To analyze the impact of season, temperature, and storage on various Physico-chemical properties of honey.

5. Review of related literature and research:

The present literature review deals with the composition of honey of various botanical and geographical origins.

- Honey is a natural product produced from the nectar and exudation of plants by the honeybees, *Apis mellifera* **(Alvarez-Suarez *et al*. 2010)**.
- The natural honey has been reported to contain about 200 substances, which consist of a not only highly concentrated solution of sugars, but also the complex mixture of other saccharides, amino acids, peptides, enzymes, proteins, organic acids, polyphenols, carotenoid like substances, vitamins, and minerals **(Gheldof *et al*. 2002b; White 1975)**.
- Sugars are the main constituents of honey, comprising about 95% of its dry weight **(Alvarez-Suarez *et al*. 2010)**.
- While glucose (31%) and fructose (38%) are the dominant constituents, about 25 different sugars have been detected. The actual proportion of glucose to fructose in any particular honey depends largely on the source of the nectar. The average ratio of fructose to glucose is 1.2:1 **(White 1975).**
- Saccharose (sucrose) is present in honey (approximately 1% of its dry weight). However, this level can be increased if the beekeeper has over-fed the bees with sugar during the spring. The level of organic acids in honey is relatively low and about 18 organic acids have been detected **(Nanda et al. 2003).**
- Most of the acidity present in honey is added by honeybees. Gluconic acid, the predominant organic acid of honey, is the product of glucose oxidation, present at 50-fold higher levels than other acids **(Cherchi et al. 1994).**
- The mineral content varies from about 0.04% in pale honey to 0.2% in some dark honey samples and is dependent on the type of soil in which the original nectar-bearing plant is located. Investigations have shown that a wide range of trace elements are present in honey, including Al, Ba, Bi, Co, Cr, Mo, Ni, Pb, Sn, Ti, as well as macro minerals like Ca, Cu, Fe, K, Na, Mg, Mn, Zn. Among them, the main mineral element is potassium while copper is present in the lowest amount (Nanda et al. 2003). Very minute quantities of Vitamins such as thiamine (B1), riboflavin (B2), pyridoxine (B6), and ascorbic acid (C) have also been reported to be present in honey samples **(Nanda et al. 2003).**

6. The Gap in existing Research

- It is clear from the above literature review that nobody has considered the study of Physical and Biochemical parameters of honey in western Maharashtra.
- No one seems to have studied the effect of different varieties of flowers, climate, and seasons on the quality and quantity of honey available in western Maharashtra.
- Although some of the researchers have considered only Physical and Biochemical characteristics some considered antioxidant and antimicrobial activity separately like workpiece material in other regions and countries but not specifically in western Maharashtra.

7. Assumptions:

- Special training is required to prevent bees from being harmed and to collect honey samples properly.
- Formal training is required for the analysis of samples collected in the laboratory.

8. Null hypothesis:

- There will not be a significant difference between pre and post-test having subjective self-concept.

9. Research methodology

- Six different districts (Sangli, Satara, Kolhapur, Solapur, Pune, and Nashik) have been selected for honey sampling.
- Ten samples from each of the six districts in western Maharashtra will be drawn.
- Honey samples will be collected from beekeepers at the time of visiting the six districts. Samples will be confirmed free of contamination.
- The colony from which the honey samples are collected should be free of disease.
- Samples will be collected in pre-labeled (identifiable) bottles.
- Honey samples will be stored in airtight bottles at room temperature.
- Mark the bottle as follows −
    1. Collection date
    2. Collection location
    3. District
    4. Bee species
    5. Brand/Beekeeper Name
    6. Amount required to collect a honey sample

10.Data analysis tools / statistical tools

- Analysis of variance according to Duncan's test was done to compare the variables of different kinds of honey collected from western Maharashtra.
- Correlation coefficient test was applied to test the association between the polyphenol content and the antioxidant activity of the kinds of honey.

11.Scope, limitation, and delimitation:

A. **Scope:**

- The results of the research are applicable for districts of western Maharashtra.

B. Limitation:

- composition of honey may be changed according to the flowers of different types of plants.
- Pesticides used by farmers, contaminated water, food may affect the quality of honey.

C. Delimitation:

- The researcher has selected only districts of western Maharashtra.
- The researcher selected Sangli, Satara, Kolhapur, Solapur, Pune, and Nashik districts

12.Importance of study/ Society application:

A. Significance of the study:

- Understand the Physical, and Biochemical properties of honey samples.
- Understand the Antioxidant and Antimicrobial properties of honey.
- we can also get an idea of toxic residues of pesticides, antibiotics, and heavy metals in honey.
- we can get an idea about regional differences in the physical, and Biochemical properties of honey.
- It will also be useful to study the effect of plant flowers on the quality of honey.
- It is also possible to note the impact of the surrounding environment and the season on the quality and quantity of honey production.

B. National status:

The honey analysis is important -

- to check the quality of the product to the honey producers.
- to determine if there are any physical, chemical, and biological contaminants or adulteration, or risk in honey production.
- to assure their honey consumers about the quality of their honey products
- to deliver a good quality of honey to the buyers by strictly analyzing the honey.

C. International status:

The honey analysis is important -

- to know the importance of honey components from a safe and healthy point of view.
- to create a distinct identity for honey production nationally and internationally.
- to build an active honey analysis network at the local, national, or international level, it is necessary to understand the process of honey analysis.
- If honey is properly analyzed and people's faith is won, we can ship it all over the world.

13.Chapterisation :

- Chapter 1:Introduction
- Chapter 2: Review of related literature and research
- Chapter 3: Methodology
- Chapter 4: Data collection and interpretation
- Chapter 5: Results and Discussion
- Chapter 6: Summary conclusion and findings

14.Year-wise Plan of work and targets to be achieved:

Year	Months	Goals	Activities
1	**1-3**	• Writing the introduction and structure • **(**Discussions with the supervisor	• Reading each week • Continue with learning Honey analysis • **Write first chapter (introduction)**
	3-10	• Review of Literature and development in the subject • (Previous work done in the relevant area) • **(**Discussions with the supervisor	• Reading each week • (Identifying gaps in the literature/data) • **Write second chapter (literature review)**
	10-12	• Methodology and research questions section **(**Discussions with the supervisor	• Reading each week • **Write third chapter (Research Methodology)**
2	**1-3**	• Preparing for fieldwork in Western Maharashtra • Revising methods and focus	• Plan of fieldwork • Obtaining permission for research in different beekeeping centers and collect the honey sample

	3-6	• Spending time in Western Maharashtra for collating primary data	• Conducting appropriate interviews and surveys by working with local beekeepers and meeting with government and local officials • **Write fourth chapter (Data collection and interpretation)**
	6-9	Writing section one of results and discussion (main body of the thesis)	• Nature of chemical composition of honey, Honey production, and selling position of Western Maharashtra in the Indian market • Economic and environmental Effects on honey extraction center. • Cultural identity, philosophical debate • **Write fifth chapter (section one of results and discussion):** •
	9-12	• Writing section two of the results and discussion (main body of the thesis)] • **(**Discussions with the supervisor **)**	• Outline the relationship between climate change, flowering herbs, shrubs, and trees • Climate and types of plants flower change in nature of honey. what are the impacts? • The response at local, national, regional, and international levels **Write fifth chapter (section two of results and discussion):**
3	**1-3**	• Writing section three of results and discussion	• Discussion on the chemical composition of honey, • Findings of fieldwork research – who is most impacted? What is the response and what is needed? Local power networks and local people's role in climate activism • **Write fifth chapter (section three of results and discussion):**
	3-6	• Conclusion • **(** Discussions with the supervisor **)**	• **Write sixth chapter Summary conclusion and findings)**
	6-9	• Proofreading	• Revise and fill gaps
	9-12	• Final discussions with the supervisor	• Any interviews to follow up with? • Ensure the thesis is up to date with all current literature and data • Update rewrites as needed

15.Budget :

Estimate	First	Second	Gross Total
Books and Journals	10,000	10,000	20,000
Contingency (including special needs)	15,000	15,000	30,000
Equipment's for analysis	70,000	70,000	1,40,000
Field Work and Travel	20,000	20,000	40,000
Hiring Services	10,000	10,000	20,000
Total	**1,25,000**	**1,25,000**	**2,50,000**

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